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Mutagenicity of Diesel-Exhaust Particle Extracts Collected under Smog-Chamber Conditions Using the 'Salmonella typhimurium' Test System

(U.S.) Health Effects Research Lab.
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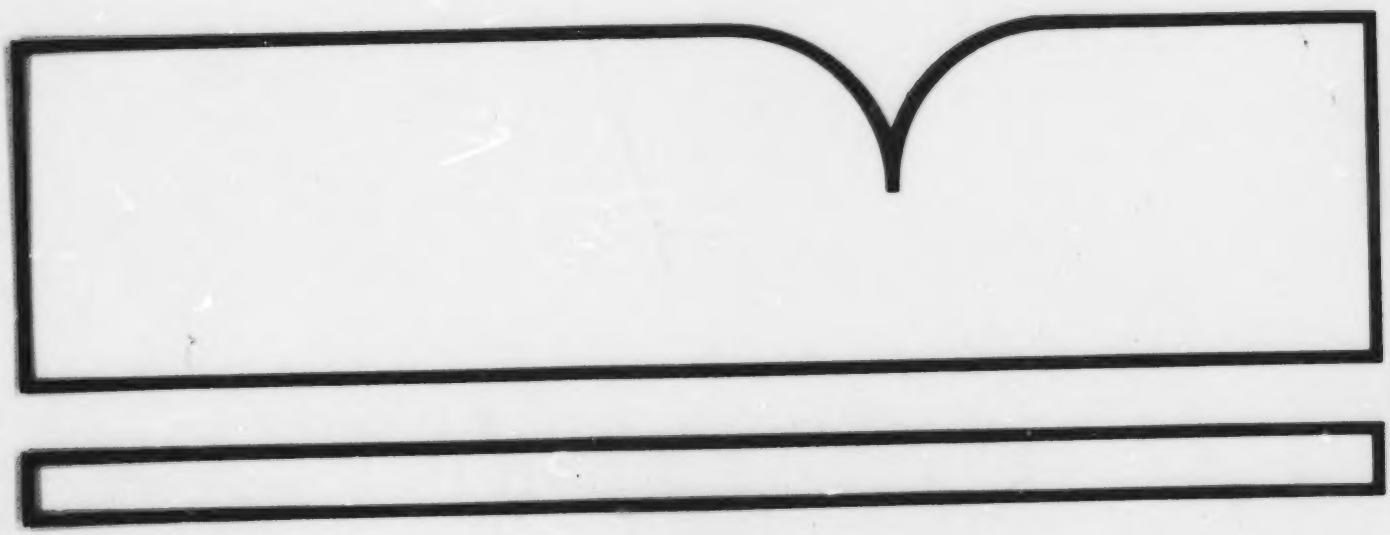

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16. ABSTRACT

This study was designed to detect the effect that different environmental conditions have upon diesel-exhaust organics. In this study, diesel-exhaust was injected into the Calspan smog chamber under different conditions, and the resulting particles were collected upon Pallflex glass-fiber filters. After extraction from the particles with methylene chloride, the organics were solvent exchanged to dimethyl sulfoxide and tested in the Salmonella typhimurium plate-incorporation test. Results demonstrate that the irradiation of propylene, SO_2, NO and NO_2 produces ozone and a mutagenic moiety. Unless another mitigating factor (e.g., ozone) was present or formed, irradiation did not alter the mutagenic response of the organics. The production or injection of ozone into chamber tended to reduce the mutagenic response of the collected organics. In summary, this study demonstrates that ambient conditions can alter the mutagenic response of diesel-exhaust organics.

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THE MUTAGENICITY OF DIESEL-EXHAUST PARTICLE EXTRACTS COLLECTED UNDER SMOG-CHAMBER CONDITIONS USING THE *Salmonella typhimurium* TEST SYSTEM

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Summary

This study was designed to detect the effect that different environmental conditions have upon diesel-exhaust organics. In this study, diesel exhaust was injected into the Calspan smog chamber under different conditions, and the resulting particles were collected upon Pallflex glass-fiber filters. After extraction from the particles with methylene chloride, the organics were solvent exchanged to dimethyl sulfoxide and tested in the *Salmonella typhimurium* plate-incorporation test. Results demonstrate that the irradiation of propylene, SO_2, NO and NO_2 produces ozone and a mutagenic moiety. Unless another mitigating factor (e.g., ozone) was present or formed, irradiation did not alter the mutagenic response of the organics. The production or injection of ozone into chamber tended to reduce the mutagenic response of the collected organics. In summary, this study demonstrates that ambient conditions can alter the mutagenic response of diesel-exhaust organics.

Previous work has shown that extracts from diesel-exhaust particles are mutagenic when tested in the *Salmonella typhimurium* plate-incorporation assay [1]. Because laboratory dilution tunnels were used for particle sampling, the effects of ambient-like conditions were unknown. Smog-chamber conditions can simulate ambient conditions such as sunlight, relative humidity, and temperature; therefore, in this study, diesel-exhaust particulates and organics were exposed to various smog-chamber conditions at the time of diesel-particle generation.

TABLE 1

EXPERIMENTAL CONDITIONS FOR THE EXPOSURE AND COLLECTION OF THE VARIOUS SAMPLES IN THE STUDY OF SMOG-CHAMBER EXPOSED DIESEL ORGANICS

Expt. No.	Sample source: variable	Sample number (ARBX-79-)	Expt. proc. [a]	RH (%) [b]	Irrad. [c]	Gases (ppm) [d]				Prop. [e] spike (ppmC)	Exposure time (h)
						SO_2	NO	NO_2	O_3max		
1	Prop/SO_2/NO_x: aerosols	0018	—	19	I	0.52	0.20	0.22	*	3.0	6.0
2	Diesel: unirradiated	0020	U	72	No	0.89	>5.0	1.1	<0.001	—	0.5
	Diesel: unirradiated	0021	U	72	No	0.89	>5.0	1.1	<0.001	—	6.0
3	Diesel: irradiated	0029	U	56	I	1.2	>5.0	*	*	—	0.5
	Diesel: irradiated	0030	U	56	I	1.2	>5.0	*	*	—	6.0
4	Diesel: injected O_3	0022	C4	71	No	0.21	<0.001	0.60	0.65	—	6.0
5	Diesel: filtered	0023	F	67	No	1.10	>5.0	1.0	*	—	0.5
	Diesel: tailpipe	0024	T	—	No	—	—	—	—	—	—
6	Diesel: diluted	0025	C4,D(15)	41	I	0.60	0.20	0.04	0.012	3.0	6.0
7	Diesel: toluene	0026	C4,D(25)	53	I	0.60	0.09	0.10	0.24	—	6.0
8	Diesel: low SO_2	0027	C4,D(25)	57	I	0.06	0.06	0.09	0.54	3.0	6.0
9	Diesel: normal SO_2	0028	C4,D(25)	63	I	0.56	0.09	0.08	0.53	-	6.0
10	Diesel: tunnel (D-1)	0504	X	—	No	—	—	(1.38)	—	—	—
11	Diesel: tunnel (D-2)	0505	X	—	No	—	—	(1.38)	—	—	—

[a] Experimental procedures: U, emissions injected directly into chamber without filtering or diluting; C = CVS dilution used; D, dilution of system with absolute filtered rural air (number of minutes); F, emissions filtered at tailpipe and collected from chamber; T, emissions filtered and collected at tailpipe; X, laboratory dilution-tunnel studies.

[b] Relative humidity in percent.

[c] Absence (no) or presence (I) of irradiation.

[d] Initial concentration only.

[e] Initial propane spike.

[f] Dilution-tunnel value.

* Equipment failure.

The *Salmonella typhimurium* plate-incorporation assay as developed by Ames and associates provides an inexpensive, rapid and sensitive assay for detecting mutagenic substances [2]. This system also provides a means of screening small samples and samples composed of a complex mixture of organics.

Materials and Methods

Samples. The smog-chamber samples were generated using a Mercedes Benz 2400 4-cylinder diesel automobile run on an engine dynamometer using the Highway Fuel Economy Test driving cycle (HWFET) and were collected at the Calspan Smog-Chamber facilities, Ashford (NY). The Calspan facility consists of a cylindrical chamber 9.14 m (30 ft.) in diameter and 9.14 m high enclosing a volume of 600 m^3 (20 800 cu. ft.). The wall is 1.25-cm thick stainless steel. The interior surface is coated with a fluoroepoxy-type urethane developed by the Naval Research Laboratory, Washington (DC). The material is similar to FEP Teflon in surface energy and reactivity properties. Illumination is provided by 28.6 kW of fluorescent blacklight and sunlamps installed inside 24 lighting modules and arranged in 8 vertical channels attached to the chamber wall. Each lighting module contains two 40-W sunlamps, eight 85-W high output black lamps, and two 215-W specially produced black lamps. The modules are covered with 0.5-cm thick Pyrex glass, air-cooled and sealed from the chamber working volume to minimize contamination. Conditions varied according to a host of parameters, including: (1) presence or absence of UV irradiation, (2) sampling location, (3) length of chamber exposure, (4) presence of ozone, (5) presence or absence of primarily diesel aerosols, and (6) addition of reactive hydrocarbons such as propylene or toluene. Table 1 gives a summary of the samples collected. The first baseline study was the forming of aerosols upon the addition of propylene, SO_2, NO and NO_2 to the smog chamber without diesel exhaust. Unirradiated baseline studies are samples 20 and 21, while irradiated baseline studies are samples 29 and 30. Samples, 25, 26, 27 and 28 also were irradiated for 6 h with fluorescent blacklight and sun lamps. All other samples were dark, unirradiated samples. In addition to the samples collected from the smog chamber, a tail-pipe sample and 2 experimental dilution-tunnel samples were collected. The tail-pipe sample (sample 24) was collected directly from the automobile tail pipe at near exhaust temperatures. The exhaust gases and particles that passed through the tail-pipe filter entered the smog chamber and were later collected as sample 23. Expt. 10 contained the dilution-tunnel samples that were collected by a standardized EPA protocol [3] at the Environmental Protection Agency Laboratory, Ann Arbor (MI) under the direction of Mr. Tom Baines. Among other variables tested were: (1) artificially supplied ozone (sample 22), (2) the effect of diluting with filtered ambient air (sample 25), and (3) a comparison of the effect of a low level of SO_2 and a higher level of SO_2 (samples 27 and 28). Measured variables including peak ozone levels are given in Table 1.

The particles were collected on Pallflex T60A20 glass-fiber filters during June of 1979, and the description of the chamber and method of collection have been published [4]. The filters were folded, sealed in wax paper, placed in

a manila envelope and stored refrigerated or on dry ice prior to extraction. The mutagenic activity of diesel particles stored under these conditions has been shown to be stable (1). All samples were extracted using methylene chloride in a soxhlet apparatus, concentrated to dryness under nitrogen, and redissolved in dimethyl sulfoxide (DMSO) to give a final concentration of either 2.0 or 2.5 mg of organic residue per ml of DMSO.

Bioassay method. The DMSO solution of diesel organics was assayed in the *Salmonella typhimurium* plate-incorporation assay [1,2]. When possible, a minimum of 5 doses applied in triplicate were tested for each sample. Samples that are reported together were run simultaneously within the particular bacterial strain cited. The extracts were examined in the presence and absence of Aroclor 1254 induced rat-liver S9 prepared according to the method of Ames et al. [2]. The slope (Table 2) for each dose–response curve was calculated from a linear regression line fitted to the linear portion of the curve.

TABLE 2

SLOPE OF THE MUTAGENIC RESPONSE CURVE OF SMOG-CHAMBER SAMPLES

Sample number (ARBX-79-)	S9 [a]	Slope [b]			
		TA100	TA1537	TA1538	TA98
0018	—	1.35	NT	2.86	NT
	+	1.59	0.42	3.79	1.60
0020	—	11.44	2.32	3.87	10.87
	+	8.91	3.51	5.67	18.13
0021	—	14.75	2.28	5.39	17.74
	+	9.55	3.97	6.86	13.41
0022	—	3.62	1.29	2.58	7.28
	+	3.66	1.35	3.01	7.74
0023	—	14.47	1.94	4.11	15.03
	+	9.20	2.99	5.22	13.23
0024	—	15.53	11.35	NT	31.26
	+	15.56	16.77	NT	56.13
0025	—	3.38	0.54	NT	2.95
	+	5.64	1.65	NT	9.73
0026	—	2.64	0.41	NT	2.25
	+	3.82	0.81	NT	6.23
0027	—	2.39	0.29	NT	1.88
	+	4.29	1.30	NT	6.23
0028	—	2.86	0.52	NT	4.84
	+	4.35	1.29	NT	7.28
0029	—	11.73	1.65	NT	10.28
	+	5.09	3.15	NT	13.43
0030	—	9.41	2.14	3.41	11.04
	+	10.10	2.92	5.43	19.05

NT, not tested.

[a] Metabolic activation.

[b] Slope calculated for the linear portion of the dose–response curve for each sample and expressed as revertants per microgram.

Results

In order to compare potential reaction differences in dark and irradiated environments, 3 baseline studies that yielded 5 samples were conducted. In the first baseline experiment (sample 18), an artificial mixture of propylene (3.0 ppm), SO_2 (0.52 ppm), NO (0.20 ppm) and NO_2 (0.22 ppm) were added to the test chamber without diesel exhaust. Irradiation of propylene, SO_2, and NO_x did demonstrate a mutagenic response (Fig. 1 and Table 3) and could account for approx. 10—35% of the total activity of the other samples depending upon the bacterial strain used. Although the ozone analyzer malfunctioned in Expt. 1, previous experiments have demonstrated that ozone and a large variety of organics are generated with the irradiation of this simple mixture [5,6]. 2 samples were collected from 2 baseline-chamber experiments. In one experiment, a portion of the suspended particles was sampled immediately after the diesel exhaust was put into the chamber (sample 20), and the remaining particulate was given a 4-h dark exposure before collection (sample 21). The other baseline experiment also provided 2 samples in the same manner; however, the 4-h dark exposure was replaced with a 6-h irradiation (samples 29 and 30). The results of these baseline studies containing only diesel exhaust are depicted in Figs. 2, 3 and 4 in strains TA98, TA100 and TA1537, resp. Table 2 tabulates the results as slope (revertants per microgram). These baseline dark and irradiated diesel-exhaust samples, collected both immediately, and after an extended period, gave similar mutagenic responses in the Salmonella assay. No detectable ozone was generated in the baseline studies containing diesel exhaust.

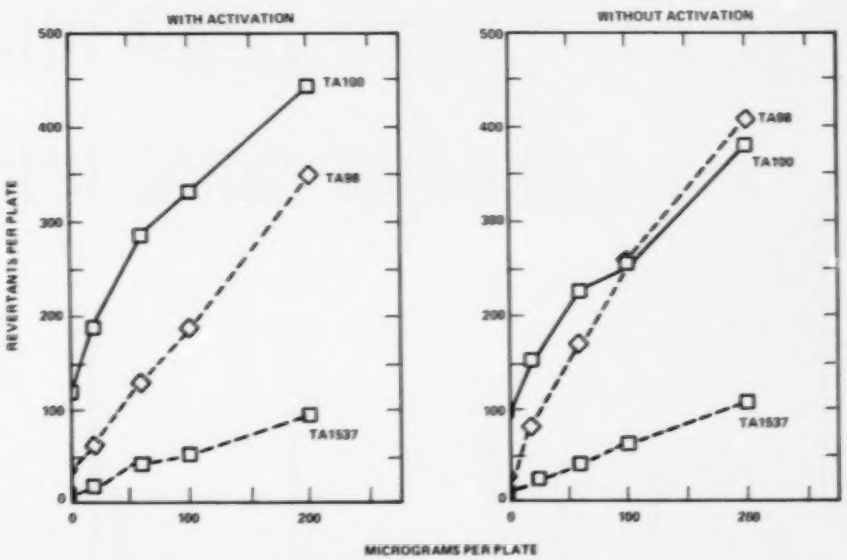

Fig. 1. Mutagenic response of an irradiated mixture of propylene, SO_2, NO and NO_2 as detected in *Salmonella typhimurium* TA100, TA98 and TA1537.

TABLE 3

MUTAGENICITY OF CONTROL AND DIESEL-EXHAUST ORGANICS EXPOSED TO SMOG-CHAMBER CONDITIONS AND TESTED IN THE *SALMONELLA TYPHIMURIUM* PLATE-INCORPORATION TEST

Compound	S9 [a]	Dose (μg)[b]	Revertants per plate for strain							
			TA100		TA1537		TA1538		TA98	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Positive control [c]	+		992.67	63.06	115.33	18.01	907.00	42.48	640.67	42.44
	—		1273.33	46.50	1133.67	175.16	405.67	11.68	259.67	29.02
Negative control										
Dimethylsulfoxide	+	100.00 μl	125.33	9.81	16.33	6.66	27.67	2.08	23.33	2.31
Dimethylsufoxide	—	100.00 μl	150.00	8.19	12.67	0.58	20.68	7.94	15.00	5.03
ARBX-79-0018										
Propylene, SO_2, NO_x	+	20.00	189.00	11.31	17.50	0.71	105.50	16.26	63.50	4.95
	+	60.00	285.50	14.85	43.00	1.41	260.00	31.11	130.00	1.41
	+	100.00	332.00	2.83	52.00	1.41	438.50	2.12	187.00	5.66
	+	200.00	443.00	15.56	94.50	23.33	785.50	60.10	350.00	9.90
	—	20.00	155.50	0.71	20.00	5.66	107.50	9.19	82.50	3.54
	—	60.00	227.00	16.97	42.00	1.41	250.50	12.02	171.50	20.51
	—	100.00	254.00	32.53	64.50	2.12	381.00	53.74	261.00	21.21
	—	200.00	381.00	52.33	109.00	1.41	587.00	9.90	409.50	12.02
ARBX-79-0020										
Baseline, nonirradiated, immediate	+	100.00	1011.00	12.73	371.50	23.33	626.50	2.12	1800.50	14.85
	+	200.00	1215.50	48.79	702.50	10.61	1144.50	28.99	2198.00	67.88
	+	600.00	960.00	77.78	486.50	4.95	765.00	38.18	2839.00	43.84
	—	20.00	547.00	15.56	48.00	4.24	120.00	46.67	467.50	12.02
	—	60.00	849.50	77.07	160.00	19.80	276.00	43.84	1101.50	21.92
	—	100.00	934.50	31.82	253.00	9.90	402.00	16.97	1599.00	118.79
	—	200.00	834.00	26.87	407.50	9.19	513.50	152.50	2200.00	5.66
	—	600.00	20.50	0.71	—	—	—	—	2130.50	53.03
ARBX-79-0021										
Baseline, nonirradiated 6-h exposure	+	20.00	432.50	7.78	66.50	7.78	155.00	11.31	596.50	76.66
	+	60.00	844.50	14.85	222.00	4.24	461.00	16.97	1421.00	106.07
	+	100.00	1070.50	10.61	462.50	43.24	843.00	59.40	2031.00	63.64
	+	200.00	1167.00	33.94	785.00	35.36	1364.00	277.19	2718.50	30.42
	+	600.00	962.00	7.07	392.50	95.46	—	—	2796.00	100.41
	—	20.00	766.00	60.81	74.00	8.49	156.00	25.46	599.50	21.92
	—	60.00	1059.50	75.66	214.00	43.84	413.00	9.90	1341.50	9.19

	—	100.00	1029.50	48.79	336.00	63.64	540.50	12.02	1778.50	43.13
	—	200.00	799.00	55.15	457.50	54.45	389.00	36.77	2280.00	131.52
	—	600.00	4.00	1.41	—	—	—	—	1996.50	103.94
ARBX-79-0022										
Injected ozone	+	20.00	242.00	22.63	24.00	4.24	75.00	12.73	174.50	13.44
	+	60.00	421.50	6.36	80.00	8.49	225.00	26.87	568.50	13.44
	+	100.00	602.50	61.52	154.50	0.71	338.50	0.71	915.00	25.46
	+	200.00	852.00	31.11	277.50	50.20	620.00	59.40	1562.50	65.76
	+	600.00	1143.00	80.61	419.00	33.94	761.00	29.70	2181.50	6.36
	—	20.00	324.50	7.78	41.00	4.24	139.50	6.36	236.00	9.90
	—	60.00	540.50	9.19	92.00	18.38	256.50	36.06	656.50	10.61
	—	100.00	718.50	14.85	141.00	56.57	386.50	70.00	964.50	31.82
	—	200.00	876.00	1.41	272.50	16.26	538.50	40.31	1471.50	68.59
	—	600.00	1020.50	19.09	345.50	17.68	807.50	38.89	1012.50	28.99
ARBX-79-0023										
Filtered exhaust	+	20.00	346.00	2.83	56.00	7.07	96.00	22.63	310.50	28.99
	+	60.00	728.50	4.95	127.50	20.51	316.00	28.28	1109.50	55.86
	+	100.00	1041.00	8.49	158.50	120.92	657.50	7.78	1702.50	241.12
	+	200.00	1331.00	29.70	640.00	14.14	1028.50	245.37	2628.50	23.33
	+	600.00	909.50	70.00	378.50	31.82	496.50	6.36	2323.50	38.89
	—	20.00	675.50	38.89	47.50	7.78	107.00	14.14	457.00	9.90
	—	60.00	1036.50	36.06	135.50	3.54	296.50	14.85	1011.00	5.66
	—	100.00	875.50	60.10	203.00	42.43	418.00	7.07	1548.00	56.57
	—	200.00	679.50	3.54	263.00	46.67	342.00	74.95	1873.50	140.71
	—	600.00	2.50	2.12	—	—	—	—	1422.50	28.99
ARBX-79-0024										
Tail-pipe sample	+	20.00	866.00	*	425.00	*	not tested		2372.00	*
	+	60.00	1020.00	*	1016.00	*			3300.00	*
	+	100.00	779.00	*	603.00	*			3253.00	*
	+	200.00	558.00	*	372.00	*			3210.00	*
	+	600.00	328.00	*	—	—			3248.00	*
	—	20.00	778.00	*	324.00	*			1736.00	*
	—	60.00	1053.00	*	686.00	*			2768.00	*
	—	100.00	805.00	*	589.00	*			2940.00	*
	—	200.00	518.00	*	405.00	*			3169.00	*
	—	600.00	3.00	*	—	—			2750.00	*
ARBX-79-0025										
Diluted, irradiated	+	20.00	297.00	*	29.00	*	not tested		189.00	*
	+	60.00	556.00	*	99.00	*			632.00	*
	+	100.00	807.00	*	177.00	*			1231.00	*
	+	200.00	1227.00	*	464.00	*			1009.00	*

TABLE 3 (continued)

Compound	S9 [a]	Dose (μg) [b]	Revertants per plate for strain							
			TA100		TA1537		TA1538		TA98	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
ARBX-79-0025										
Diluted, irradiated	+	600.00	1468.00	*	981.00	*			2724.00	*
	–	20.00	243.00	*	19.00	*			154.00	*
	–	60.00	373.00	*	67.00	*			373.00	*
	–	100.00	555.00	*	80.00	*			666.00	*
	–	200.00	811.00	*	172.00	*			1073.00	*
	–	600.00	1108.00	*	335.00	*			1779.00	*
ARBX-79-0026										
Toluene injection, irradiated	+	20.00	191.00	*	20.00	*	not tested		155.00	*
	+	60.00	417.00	*	61.00	*			402.00	*
	+	100.00	534.00	*	87.00	*			626.00	*
	+	200.00	875.00	*	195.00	*			1298.00	*
	+	600.00	1143.00	*	499.00	*			1781.00	*
	–	20.00	215.00	*	41.00	*			138.00	*
	–	60.00	312.00	*	63.00	*			336.00	*
	–	100.00	478.00	*	66.00	*			540.00	*
	–	200.00	660.00	*	116.00	*			813.00	*
	–	600.00	798.00	*	267.00	*			1377.00	*
ARBX-79-0027										
Low SO_2, irradiated	+	20.00	167.00	*	33.00	*	not tested		115.00	*
	+	60.00	440.00	*	79.00	*			303.00	*
	+	100.00	608.00	*	131.00	*			630.00	*
	+	200.00	952.00	*	311.00	*			1291.00	*
	+	600.00	1340.00	*	786.00	*			1966.00	*
	–	20.00	202.00	*	32.00	*			98.00	*
	–	60.00	391.00	*	69.00	*			262.00	*
	–	100.00	403.00	*	67.00	*			334.00	*
	–	200.00	620.00	*	129.00	*			554.00	*
	–	600.00	804.00	*	189.00	*			1161.00	*
	+	600.00	1322.00	*	768.00	*			2210.00	*
	–	20.00	259.00	*	40.00	*			154.00	*
	–	60.00	440.00	*	90.00	*			403.00	*
ARBX-79-0028										
Normal SO_2, irradiated	+	20.00	218.00	*	20.00	*	not tested		160.00	*
	+	60.00	458.00	*	99.00	*			520.00	*

	+	100.00	652.00	*	158.00	*			910.00	*
	+	200.00	965.00	*	373.00	*			1447.00	*
	—	100.00	560.00	*	110.00	*			555.00	*
	—	200.00	690.00	*	159.00	*			988.00	*
	—	600.00	980.00	*	330.00	*			1450.00	*
ARBX-79-0029										
Baseline, irradiated immediate	+	20.00	308.00	2.83	49.00	8.49	not tested		340.00	12.73
	+	60.00	673.00	94.75	136.50	9.19			1044.50	62.93
	+	100.00	912.00	59.40	248.50	23.33			1806.50	51.62
	+	200.00	1124.00	0.00	650.50	28.99			2655.00	43.84
	+	600.00	874.50	43.13	498.00	*			2690.00	49.50
	—	10.00	475.50	27.58	52.50	16.26			411.50	17.68
	—	60.00	861.00	29.70	158.00	12.73			1182.00	48.08
	—	100.00	878.50	51.62	226.00	11.31			1626.50	75.66
	—	200.00	735.50	7.78	338.00	52.33			2054.00	31.11
	—	600.00	—	—	—	—			1790.50	30.41
ARBX-79-0030										
Baseline, irradiated 6 hour exposure	+	20.00	413.00	7.07	54.50	0.71	120.00	38.18	500.00	50.91
	+	60.00	856.50	33.23	187.50	3.54	412.00	16.97	1404.50	30.41
	+	100.00	1122.00	82.02	341.00	5.66	739.50	3.54	1899.50	3.54
	+	200.00	1397.00	7.07	584.50	50.20	1068.00	152.74	2473.00	128.69
	+	600.00	1121.50	65.76	534.50	20.21	907.50	3.54	2838.00	73.54
	—	20.00	508.00	21.21	77.00	11.31	147.50	26.16	540.50	16.26
	—	60.00	992.00	2.83	220.00	15.56	356.00	80.61	1205.00	24.04
	—	100.00	1055.00	2.83	330.50	21.92	452.00	53.74	1564.00	115.97
	—	200.00	966.00	39.60	430.00	35.56	707.00	32.53	2279.50	6.36
	—	600.00	49.50	33.23	—	—	—	—	2097.00	144.25

[a] Metabolic activation.

[b] Dose as μg per plate.

[c] Positive controls: TA100 (+S9, 0.5 μg 2 AA; —S9, 1.0 μg sodium azide); TA 1535 (+S9, 3.0 μg 2AA; —S9, 1.0 μg sodium azide); TA1537 (+S9, 3.0 μg 2AA; —S9, 100.0 μg 9-AA); TA1538 (+S9, 0.5 μg 2 AA; —S9, 3.0 μg 2-NF); TA98 (+S9, 0.5 μg 2 AA; —S9, 3.0 μg 2-NF).

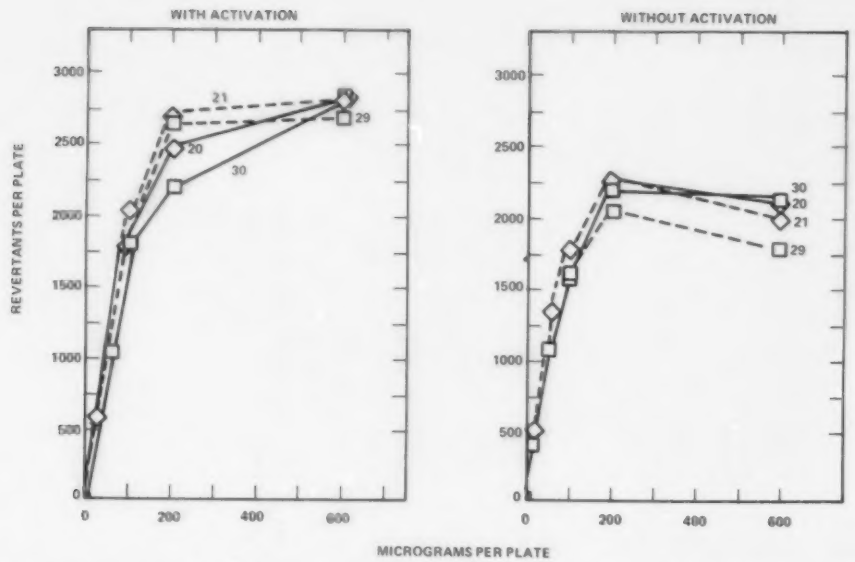

Fig. 2. Mutagenicity of diesel-exhaust organics collected immediately and after dark and irradiated exposures: *Salmonella typhimurium* TA98. 20, unirradiated (dark), immediate collection; 21, unirradiated (dark), 6-h exposure; 29, UV irradiated, immediate collection; 30, UV irradiated, 6-h exposure.

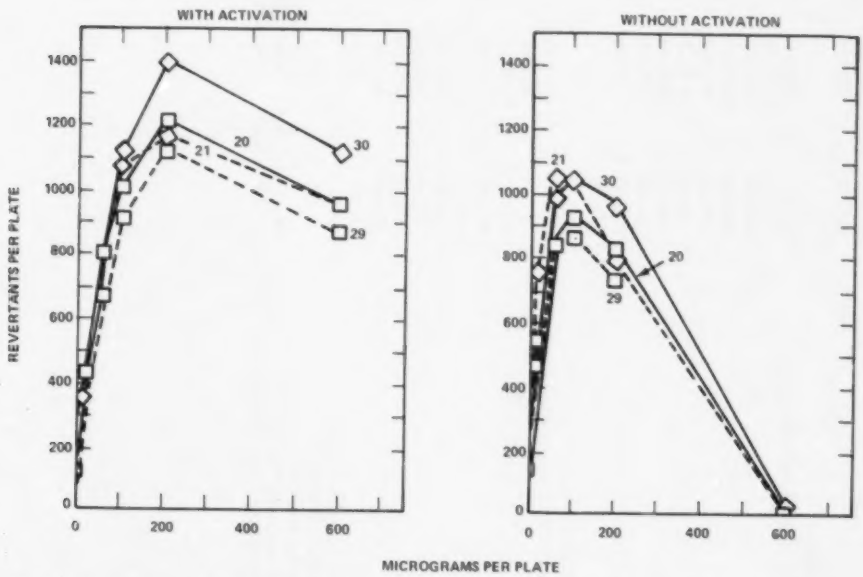

Fig. 3. Mutagenicity of diesel-exhaust organics collected immediately and after dark and irradiated exposures: *Salmonella typhimurium* TA100. 20, unirradiated (dark), immediate collection; 21, unirradiated (dark), 6-h exposure; 29, UV irradiated, immediate collection; 30, UV irradiated, 6-h exposure.

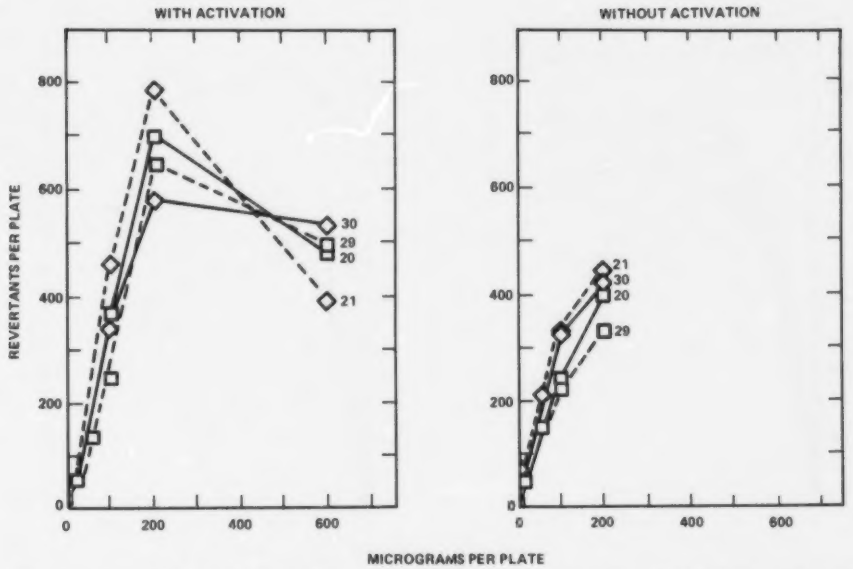

Fig. 4. Mutagenicity of diesel-exhaust organics collected immediately and after dark and irradiated exposures: *Salmonella typhimurium* TA1537. 20, unirradiated (dark), immediate collection; 21, unirradiated (dark), 6-h exposure; 29, UV irradiated, immediate collection; 30, UV irradiated 6-h exposure.

A comparison of the various non-irradiated exposures can be seen in Figs. 5, 6 and 7. As shown by these 3 tester strains, the tail-pipe samples shows a significant departure from the other non-irradiared samples. Within the linear portion of the dose—response curve, the revertants per plate for the tail-pipe samples are approximately double that of the other samples.

Figs. 8, 9 and 10 represent the results, in Salmonella of 6-h UV-irradiated samples. The modifying effects of several conditions were examined with these samples. Exhaust organics exposed to the reactive hydrocarbon propylene (sample 25) gave a consistently higher response than organics exposed to toluene (sample 26); however, there is also a 10-min difference in the dilution time between these 2 experiments. No major differences were noted in mutagenicity when the particle organics were exposed to different levels of SO_2 (samples 27 and 28). The baseline experiment in which the exhaust was irradiated and undiluted (sample 30) consistently provided more mutagenic organics than the corresponding non-irradiated sample (sample 21). Except for apparent bactericidal activity in the absence of S9, the mutagenicity of sample 30 is similar in the presence or absence of S9. The activity of samples 25—28, however, was increased by the use of the S9 fraction.

The effect of ozone is clearly seen in the experiment in which ozone was artificially added to the chamber (sample 22). Figs. 11 and 12 demonstrate how the mutagenicity of ozone-exposed organics differ from a baseline study (sample 21) in which no ozone was detected. As seen in Figs. 11 and 12 (except where bactericidal activity is prominent), the activity of sample 21 is

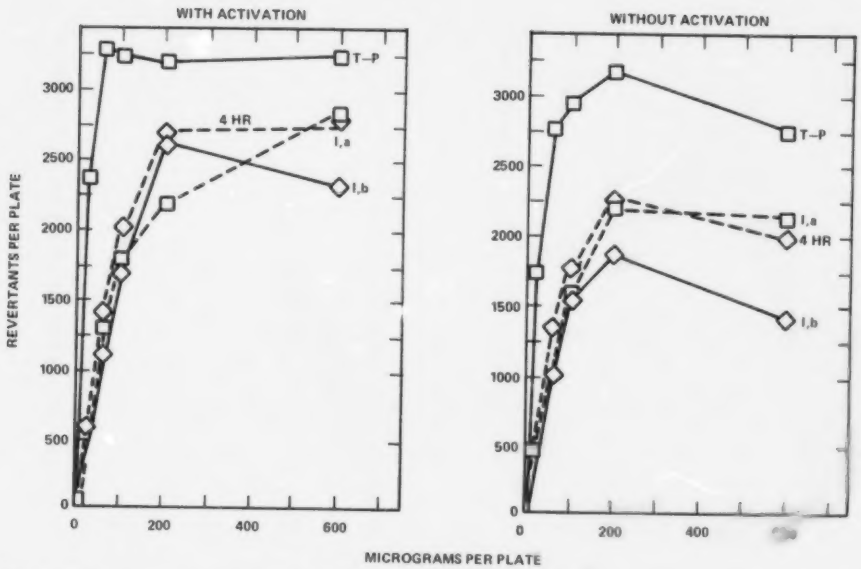

Fig. 5. Mutagenicity of tail-pipe (T-P) immediate (I) and 4-h (4-h) smog-chamber exposures of diesel exhaust organics: *Salmonella typhimurium* TA98.

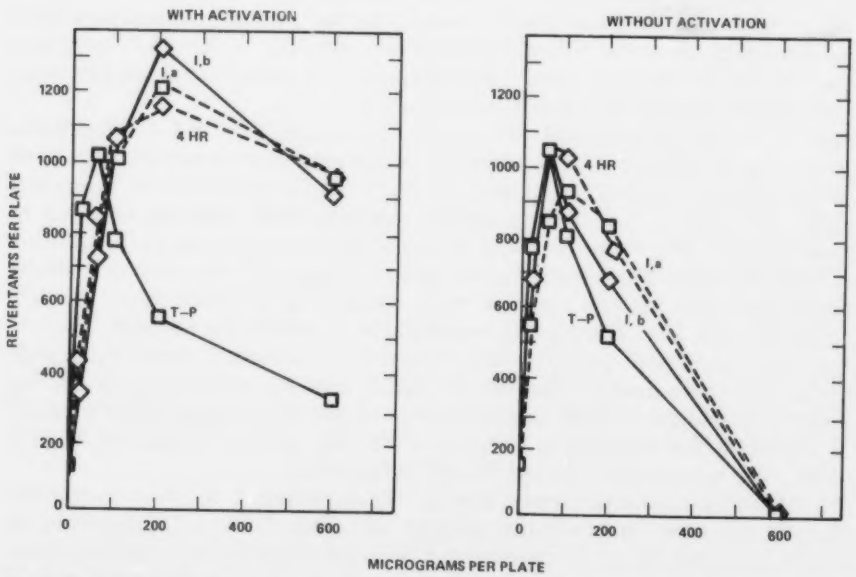

Fig. 6. Mutagenicity of tail-pipe (T-P) immediate (I) and 4-h (4-h) smog-chamber exposures of diesel-exhaust organics: *Salmonella typhimurium* TA100.

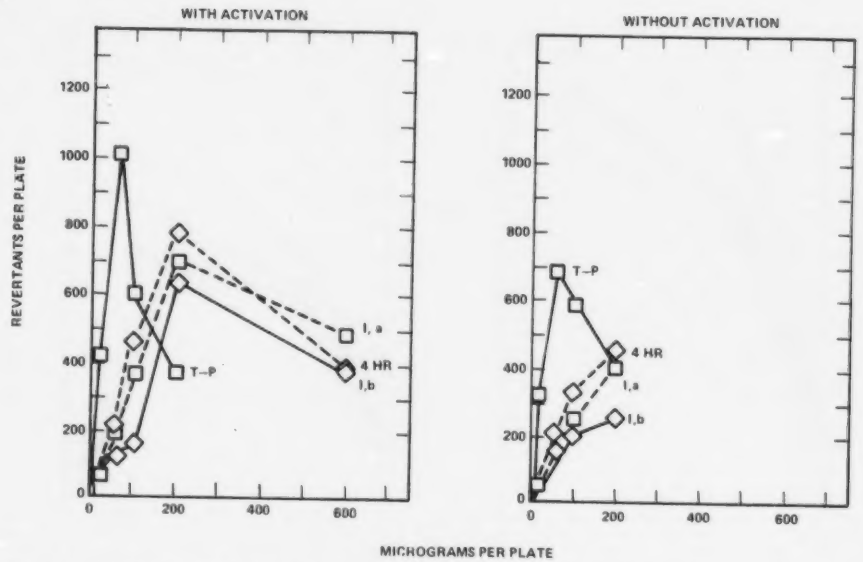

Fig. 7. Mutagenicity of tail-pipe (T-P) immediate (I) and 4-h (4-h) smog-chamber exposures of diesel-exhaust organics: *Salmonella typhimurium* TA1537.

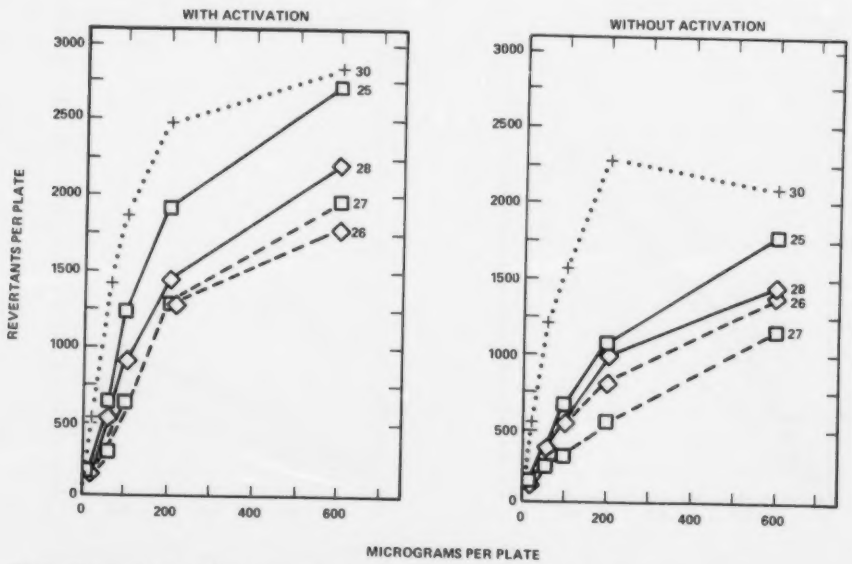

Fig. 8. Mutagenicity of diesel-exhaust organic samples irradiated under different conditions: *Salmonella typhimurium* TA98. 25, exhaust diluted with filtered air; 26, exhaust with added toluene; 27, exhaust with low SO_2; 28, exhaust with normal SO_2; 30, normal exhaust (baseline).

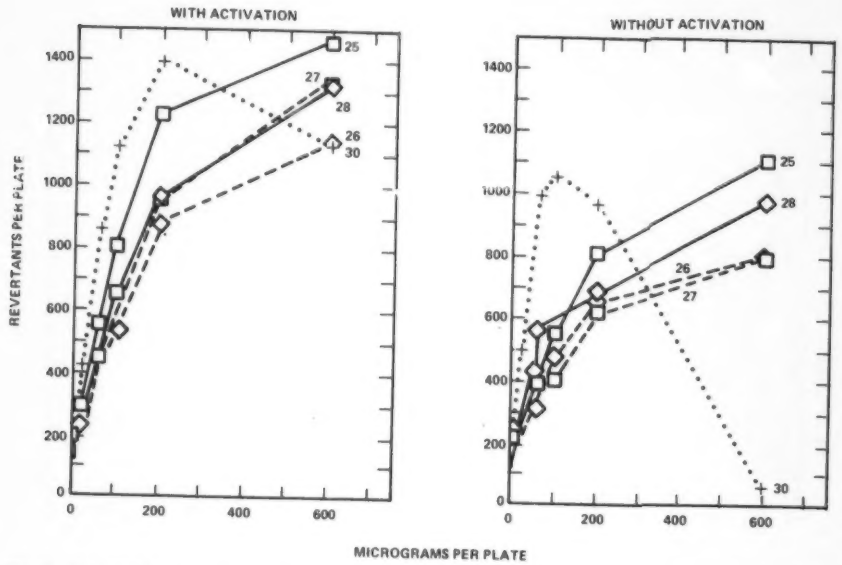

Fig. 9. Mutagenicity of diesel-exhaust organic samples irradiated under different conditions: *Salmonella typhimurium* TA100. 25, exhaust diluted with filtered air; 26, exhaust with added toluene; 27, exhaust with low SO_2; 28, exhaust with normal SO_2; 30, normal exhaust (baseline).

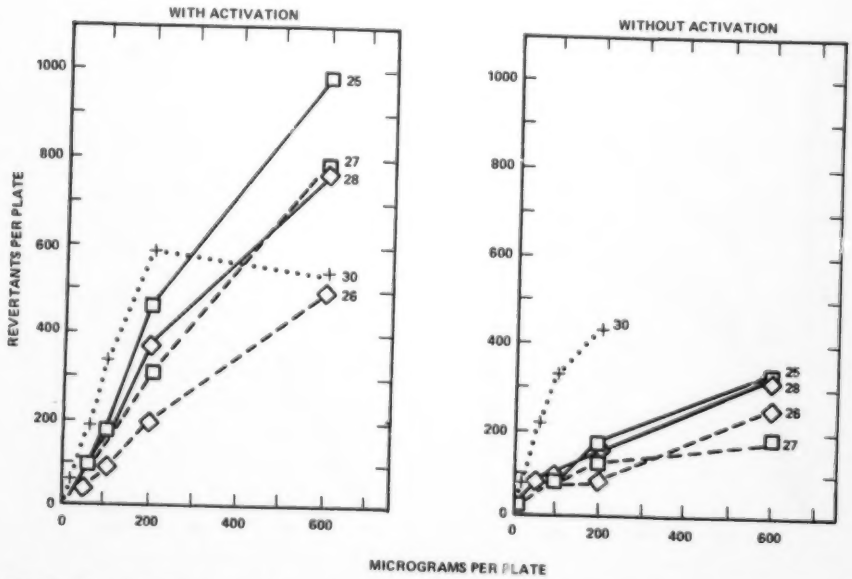

Fig. 10. Mutagenicity of diesel-exhaust organic samples irradiated under different conditions: *Salmonella typhimurium* TA1537. 25, exhaust diluted with filtered air; 26, exhaust with added toluene; 27, exhaust with low SO_2; 28, exhaust with normal SO_2; 30, normal exhaust (baseline).

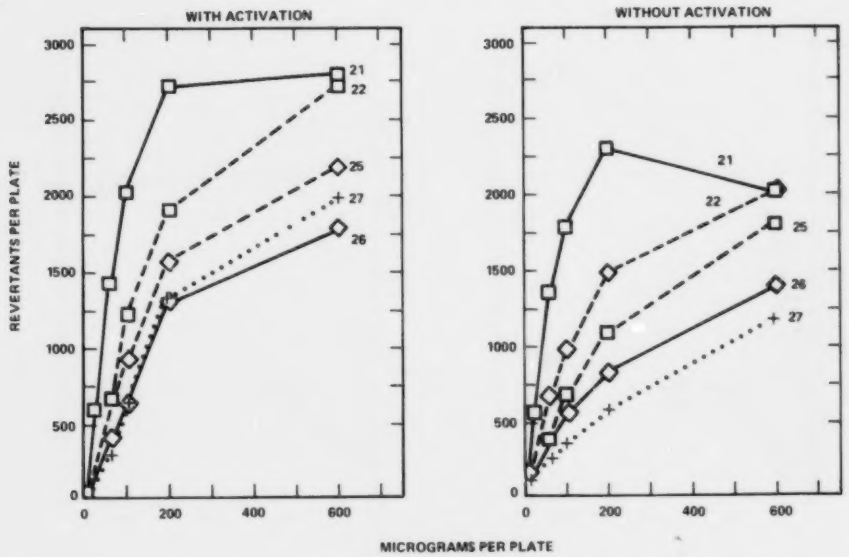

Fig. 11. Effect of ozone on the mutagenicity of diesel-exhaust organics: *Salmonella typhimurium* TA98. 21, <0.001 ppm ozone; 22, 0.65 ppm peak ozone; 25, 0.012 ppm peak ozone; 26, 0.24 ppm peak ozone; 27, 0.54 ppm peak ozone.

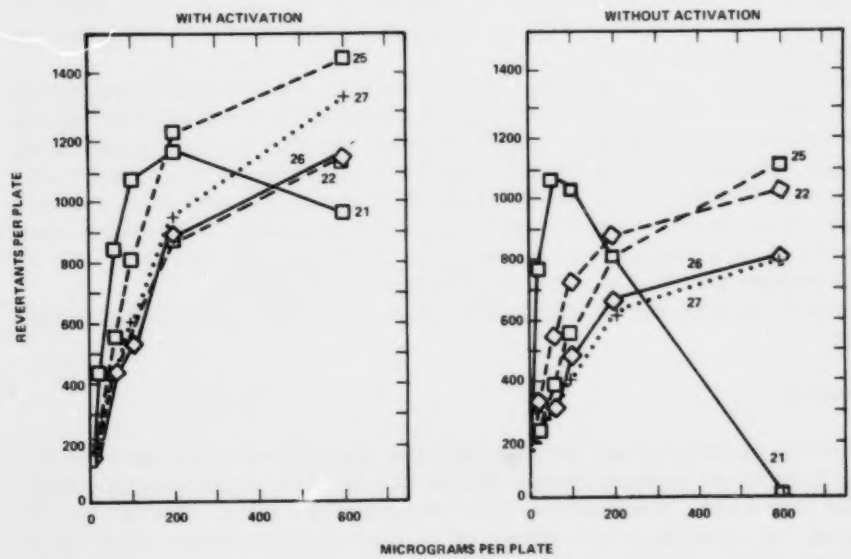

Fig. 12. Effect of ozone on the mutagenicity of diesel-exhaust organics: *Salmonella typhimurium* TA100. 21, <0.001 ppm ozone; 22, 0.65 ppm ozone; 25, 0.012 ppm ozone; 26, 0.24 ppm ozone; 27, 0.54 ppm ozone.

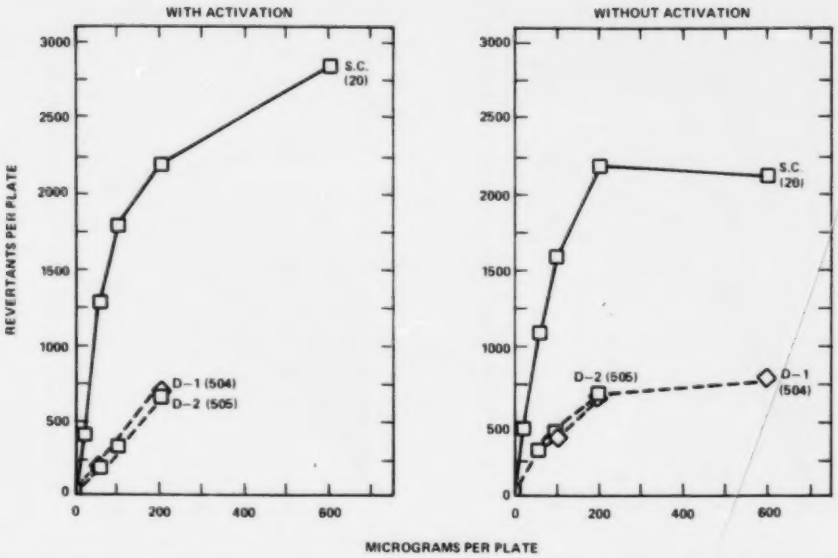

Fig. 13. Bioassay of exhaust organics extracted from dilution-tunnel collected particles and smog-chamber collected particles: TA98. S.C. smog-chamber sample 20; D-1, dilution-tunnel test number 1, sample 504; D-2, dilution-tunnel test number 2, sample 505.

nearly double that of sample 22 at each dose. Ozone also decreased the mutagenicity—bactericidal activity of samples 25, 26 and 27. The shifts in mutagenic and bactericidal activity demonstrate the chemical interaction of ozone with exhaust organics.

The 2 dilution-tunnel samples (504 and 505) are from replicate experiments with a Mercedes 300D automobile. Fig. 13 compares the bioassay results of the organics from these dilution tunnel-collected particles to particle-associated organics (sample 20) collected immediately from the smog chamber under dark conditions. Because each of these samples were generated under dark, non-irradiated, non-ozonated conditions and collected immediately, the organics were expected to have similar activity; however, a considerable difference can be noted between the smog-chamber and dilution-tunnel samples.

Table 3 provides the summary data for each sample reported within this study.

Discussion

Previous studies [1] had demonstrated that particle-associated organics collected from diesel engines through the use of a dilution tunnel were mutagenic in the *Salmonella typhimurium* plate-incorporation assay. This study was designed to determine the effect of a reactive atmosphere upon these mutagenic components, and to simulate some of the conditions found in ambient atmospheres containing diesel exhaust. The engineering procedures and associated chemistry are more fully reported elsewhere [7].

In baseline diesel-exhaust studies, where ozone was neither added nor produced, the mutagenicity of each sample was similar (Fig. 2). This indicates that mutagen production and alteration is similar under either dark or UV-light conditions, unless some other mitigating factor is introduced. Secondly, some mutagenic products are formed simply by the interaction of a simple hydrocarbon (propylene), SO_2 and NO_x gases without combustion organics being present (Fig. 1). It is understood from previous studies that the spontaneous interaction of this simple mixture creates a large variety of organics and fine aerosols [6]. The activity detected may account for 10—35% of the total activity of any smog-chamber sample in which a reactive hydrocarbon, SO_2 and NO_x exists and ozone is produced.

The sample obtained directly from the tail pipe was highly mutagenic and differed considerably from all the smog-chamber samples and previous dilution-tunnel samples. Although all samples are mutagenic, the response, and therefore those substances providing the response, varies markedly with the type of sampling. This difference, so clearly demonstrated in the tail-pipe sample, could be due to various parameters including collection temperature, concentration of exhaust gases such as NO_2 and SO_2, and rates of chemical interaction.

Samples 25—28 were exposed to ozone plus other variables. These samples produced somewhat similar dose—response curves, although some of the samples were twice as mutagenic as other samples at specific doses. It is not possible to determine from these experiments to what extent this variation is due to some of the parameters used. For example, this study demonstrates no detectable effect due to changes in SO_2 levels; however, more studies are needed to make that a definiive statement. The difference in mutagenic activity between organics produced when propylene is present instead of toluene may be real, but the 10-min difference in the fresh-air dilution times may have influenced the results. However, samples exposed to ozone gave responses that are different from the responses of non-ozonated compounds (Figs. 8—12). This difference between ozonated and non-ozonated samples is most clearly seen when mammalian activation is absent. Samples exposed to ozone also give similar responses with and without S9. Ozone obviously affected the particulate organics of these experiments in the following ways:

(1) Lowered recoverable mutagenic activity per microgram of organics. (2) Altered mutagenic activity by either transforming indirectly-acting promutagens to direct-acting compounds or inactivating a portion of the promutagens or both, and (3) Reduced the bactericidal activity of the collected organics.

Immediate (0.5-h) collections of exhaust particles from the smog chamber simulated dilution-tunnel conditions. The smog-chamber samples, however, were not typical of previously-run dilution-tunnel samples and were more mutagenic than dilution-tunnel samples (Fig. 13). Unfortunately, the automobile used in the smog-chamber studies has not, to date, been used in dilution-tunnel studies so that direct comparisons cannot be obtained.

In summary, the mutagenicity of exhaust particulate organics from diesel engines depends upon the method and conditions of collection and ambient conditions. UV irradiation without other mitigating factors was shown to have no effect upon the overall mutagenic activity of the organics collected. However, ozone reduces the mutagenicity of the organics. This study also has

demonstrated that the complex array of organics produced by the simple mixture of propylene, NO, NO_2, SO_2 and irradiation, are mutagenic. Future experiments will continue to explore the factors that affect the level of mutagenic components in ambient air due to mobile-source emissions.

Acknowledgements

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